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Carcinogenicity of Chlorination By-Products: Trihalomethanes

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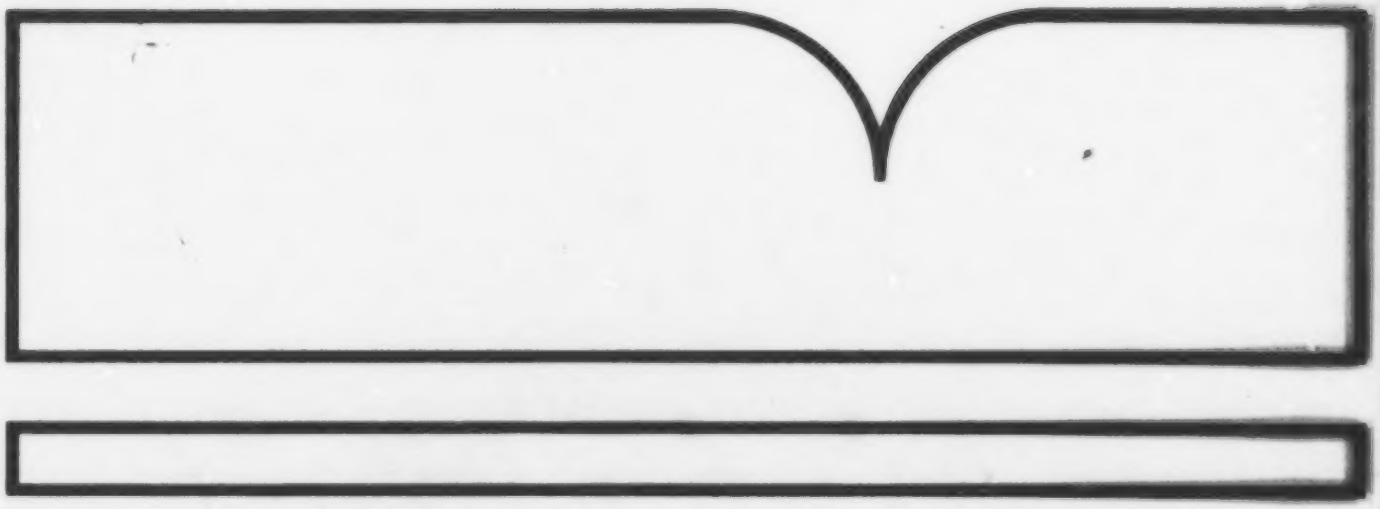

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CARCINOGENICITY OF CHLORINATION BY-PRODUCTS: TRIHALOMETHANES

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16. ABSTRACT There is insufficient evidence to indicate anything but the weakest, if any, tumor initiating activity for trihalomethanes. Chloroform in all the tests other than the low level of DNA binding did not indicate any possible tumor initiating activity. The only indication that the other trihalomethanes are tumor initiators was their marginal mutagenic activity in the Ames Salmonella Assay. The trihalomethanes would appear to possess tumor promoting activity as indicated by (1) induction of regenerative hyperplasia, (2) molecular markers such as induction of ODC and (3) the apparent weak promoting activity of chloroform in the rat liver foci bioassay. The dose-response relationship of the tumor promoting activity of chloroform could possess a threshold as indicated by the induction of ODC and regenerative hyperplasia. Therefore, the weight of the present evidence would indicate that the extrapolation model employed to estimate the low dose carcinogenic response for chloroform should be different than the model used for genotoxic carcinogens. However, it must be emphasized that confirming evidence is required to support this conclusion.

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INTRODUCTION

Trihalomethanes (THM) including bromoform and chloroform formed during the chlorination of source water have been found in finished drinking water[1-3]. Chloroform has been shown to increase the incidence of hepatocellular carcinomas and kidney epithelial tumors when administered in relatively high doses to mice and rats[4]. Using this data the U.S. EPA has proposed a maximum contaminant level (MCL) of 100 ppb THM in finished drinking water[5]. As part of the determination of the MCL, an estimation was made of the carcinogenic risk to humans of THM at the low environmental exposure. This required the extrapolation of the carcinogenicity results obtained in high dose NCI bioassays of rodents downward to the much lower dose levels of possible human exposure and then across species to humans. The downward extrapolation to lower doses required knowledge of the dose-response relationship of THM carcinogenicity which in turn is contingent on knowledge of the mechanism of action of THM.

In this communication, we review the evidence as to whether the mechanism of action of THM is genetic (initiation) or epigenetic (promotion). We also review the importance of this distinction of the mechanism of action to the estimation for THM of their human health hazard.

MECHANISM OF CARCINOGENESIS

Chemicals that can increase the incidence of cancer are called carcinogens. Two distinctly different mechanisms of action for carcinogens have been recognized which depend upon whether the action of the carcinogen is genetic or epigenetic[6,7]. The proposed genetic mechanism of chemical carcinogenesis results from the covalent reaction of the carcinogen with DNA. This alteration could produce a somatic mutation resulting in the formation of a clone (focus) of transformed cells. Thus, genetic carcinogens initiate the neoplastic progression and are called initiators. The other proposed mechanism of action for carcinogens is epigenetic and involves an alteration in the control of cellular differentiation and replication. Epigenetic carcinogens are not initiators of carcinogenesis but are promoters that decrease the time required for the appearance of "spontaneous" or chemically initiated neoplastic cells. Many carcinogens, but by no means all, possess both mechanisms of action; that is, they can both initiate and promote carcinogenesis.

Dose-Response Relationship

The crucial reason for distinguishing the mechanism of action by which a chemical induces an increased incidence of cancer is that

genetic and epigenetic carcinogens have extremely different dose-response relationships. Genetic carcinogens probably pose a risk at all dose levels. Once the carcinogen has altered the DNA (genome) and the alteration has been fixed during replication into a daughter strand, there is a finite chance that the alteration is the crucial event for the initiation of the carcinogenic progression. The binding to DNA of the genetic carcinogens acetylaminoflourene[8] in mouse and rat liver and benzo(a)pyrene[9] in mouse skin is linearly dependent in dose at levels approximately that of carcinogens in the environment. On the other hand the dose-response relationship for epigenetic carcinogens is believed to possess a threshold below which these chemicals do not pose a risk. This is because an epigenetic carcinogen has to either (1) bind to a significant number of receptors before it exerts its action or (2) cause a significant amount of cellular damage before necrosis and regenerative hyperplasia occurs. Steroids such as estrogens and contraceptive drugs are tumor promoters that possess epigenetic activity[10,11]. Their mechanism of action appears to require binding to cytoplasmic and nuclear receptors[12]. This receptor binding exhibits a threshold and requires a significant number of receptor sites to be occupied before their activity is manifested. Drug and chemically induced cytotoxicity also exhibits a threshold below which there is no necrosis and regenerative hyperplasia[13]. The necrosis, regenerative hyperplasia and tumor promotion of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) in mouse skin are exhibited at a threshold level of the chemical[14,15]. The knowledge

of the mechanism of action by which a chemical induced an increase in the incidence of cancer is therefore crucial for the estimation of the carcinogenic risk at dose levels that might be below a threshold level.

Reversibility

Another important distinction between genetic and epigenetic carcinogens is reversibility[6,7]. Genetic effects are irreversible whereas epigenetic effects are reversible. Tumor promotion requires a continuous or repeated administration of the promoter. If the promotion regimen is terminated prior to the appearance of cancer the effect of the promoter for the most part is reversed.

Initiation/Promotion Assays

Tumor initiators and promoters can be distinguished in short-term initiation/promotion assays (Figure 1). The administration alone of either the initiator or the promotion regimen does not result in an increase incidence of cancer; however, when the initiator is administered followed by the promotion regimen there does result in an increase incidence of cancer. To test a substance for initiating activity, the substance is administered followed by a known promotion

regimen, while to test for promoting activity, an initiator is administered followed by repeated administration of the substance being tested for promoting activity.

In the next two sections of this manuscript we review the evidence of whether the mechanism of action of THM is genetic or epigenetic. The evidence described is taken from the scientific literature and from unpublished and preliminary results from our laboratory.

EVIDENCE FOR GENETIC EFFECT

Mutagenesis

Chloroform has been reported to be non-mutagenic in (1) the Ames Salmonella/microsome assay[16], (2) E. coli K 12 for basepair substitution[17] 1976 and (3) 8-azaguanine locus in Chinese hamster lung fibroblast in cell culture[18]. Bromodichloromethane, dibromochloromethane and bromoform were weakly mutagenic in Ames TA 100 Salmonella without metabolic activation[16].

DNA Binding and Damage

The binding of bromoform and chloroform to liver and kidney DNA in mice and rats was determined and compared to the binding of dimethylnitrosamine (Table 1). Dimethylnitrosamine is a genetic carcinogen

that induces cancer in the liver and kidney by methylating DNA[19]. The binding of chloroform in rats to liver and kidney DNA was approximately 0.1% the binding of dimethylnitrosamine to rat liver DNA. We were unable to detect any binding to DNA of chloroform in mouse liver and bromoform in rat liver. Reitz et al[13] have reported for chloroform, relative to dimethylnitrosamine, similar low levels of binding to DNA in mouse liver. Petzold and Swenberg[20] have reported that chloroform does not damage rat liver and kidney DNA as determined by the alkaline elution technique. Dimethylnitrosamine caused extensive damage to DNA. It appears that the extent to which THM bind and damage DNA is negligible compared to genetic carcinogens.

Initiation Bioassays

Chloroform was tested in two short-term initiation bioassays. In a continuing test of chloroform in the mouse skin initiation bioassay[20], preliminary results were reported by Slaga and Daniel[22]. Groups of 40 female Sencar mice each were topically administered chloroform at a dose of either 0, 28, 240 or 280 mg/mouse and promoted with TPA (4 ug/mouse, once a week) for 23 weeks[21]. Chloroform did not increase the incidence of papillomas (Table 2).

Bromoform and chloroform were tested for initiating activity in the rat liver foci bioassay (Figure 2)[23,24]. Bromoform (0.8

mmole/kg) or chloroform (1.5 mmole/kg) were administered orally in tricaprylin to male rats 20-22 hours after they had received a partial hepatectomy which consisted of removal of about 67% of the organ or a sham operation (Table 3). Diethylnitrosamine (0.5 mmole/kg) administered orally was the positive control and tricaprylin the negative control. Three days later, the rats started to receive as the promotion regimen, 500 ppm sodium phenobarbital in their drinking water for a total of 47 days. Six days after the termination of the phenobarbital regimen, the animals were sacrificed. Cryostatic sections of the livers were obtained and stained for gamma glutamyltranspeptidase (GGTase) by the procedure of Rutenburg et al[25]. An increased incidence of GGTase-positive foci would be indicative of initiating activity; however, neither bromoform or chloroform increased the incidence of GGTase-positive foci above the solvent control (tricaprylin).

EVIDENCE FOR PROMOTING ACTIVITY

Regenerative Hyperplasia

Chloroform has long been known to be toxic to the liver and kidney, which are also the organs in which chloroform increased incidences of tumor formation[4,26,27]. Hepatotoxicity included fatty

degeneration, glycogen depletion, vacuolation, welling and necrosis. This severe tissue damage has been reported to result in regenerative hyperplasia[13] that could accelerate the neoplastic progression by an epigenetic mechanism. This proposed epigenetic increase of the incidence of tumor formation is called promotion.

Molecular Markers for Tumor Promotion

Ornithine decarboxylase (ODC) induction is an apparent prerequisite and is a proposed molecular marker for tumor promotion in skin[7] and liver[28]. Many hepatic tumor promoters including phenobarbital[29], aroclor-1254[29], steroids[30], contraceptive drugs[30] and halogenated benzenes[29] induced ODC levels. The dose-response relationship of the induction of ODC in rat liver appeared to contain a threshold (Figure 3). This would indicate that a safe dose exists for the tumor promoting activity of chloroform. We have also determined that the other THM, bromodichloromethane, chlorodibromomethane and bromoform are also potent inducers of ODC activity[30].

Promotion Bioassay (Figure2)

Chloroform was tested for promoting activity in the rat liver foci bioassay (Table 4)[5]. Male rats were initiated with diethylnitrosamine (0.5 mmole/kg) administered by gavage. Three days later, some of the rats received intra-gastrically either chloroform (1.5 mmole/kg)

or tricaprylin (solvent control) twice weekly for a total of 53 days. Other rats received 500 ppm sodium phenobarbital in their drinking water for the same length of time (positive control). Four days after the termination of the promotion regimen the rats were sacrificed. Cryostatic sections of the livers were obtained and scored for GGTase-positive foci. Neither diethylnitrosamine administered to non-promoted rats or chloroform administered to non-initiated rats resulted in a statistically significant increase in the incidence of GGTase-positive foci (table 4). A slight but significant increase in the incidence of GGTase-positive foci compared to the solvent treated control group was present in the rats that received the initiating dose of diethylnitrosamine followed by the promotion regimen of chloroform. However, the incidence of GGTase-positive foci in the group that received both the diethylnitrosamine and chloroform was not significantly different from the group treated with either diethylnitrosamine or with chloroform. Therefore, the results of the promotion assay would indicate a weak promoting activity for chloroform in rat liver that requires further confirmation.

CONCLUSIONS

There is insufficient evidence to indicate anything but the weakest, if any, tumor initiating activity for trihalomethanes. Chloroform in all the tests other than the low level of DNA binding did not indicate any possible tumor initiating activity. The only

indication that the other trihalomethanes are tumor initiators was their marginal mutagenic activity in the Ames Salmonella Assay.

- The trihalomethanes would appear to possess tumor promoting activity as indicated by (1) induction of regenerative hyperplasia, (2) molecular markers such as induction of ODC and (3) the apparent weak promoting activity of chloroform in the rat liver foci bioassay.

- The dose-response relationship of the tumor promoting activity of chloroform could possess a threshold as indicated by the induction of ODC and regenerative hyperplasia. Therefore, the weight of the present evidence would indicate that the extrapolation model employed to estimate the low dose carcinogenic response for chloroform should be different than the model used for genotoxic carcinogens. However, it must be emphasized that confirming evidence is required to support this conclusion.

ACKNOWLEDGMENTS

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TABLE 1. BINDING OF TRIHALOMETHANES TO DNA[a]

CHEMICAL	SPECIES	ORGAN	BINDING INDEX[b] CsCl
CHLOROFORM	RAT	LIVER	0.017
		KIDNEY	0.0055
	MOUSE	LIVER	NOT DETECTED
BROMOFORM	RAT	LIVER	NOT DETECTED
DMN[c]	RAT	LIVER	11.4

[a]From Reference Pereira et al.[24]

[b]Binding Index = $\frac{\text{PMOLE BOUND/MG DNA}}{\mu\text{MOLE/KG BD. WT.}}$

[c]DMN = Dimethylnitrosamine

TABLE 2. MOUSE SKIN INITIATION ASSAY FOR CHLOROFORM[a,b]

Chemical	Dose mg/Mouse	Papillomas/Mouse at 23 weeks	% of Mice with tumors at 23 weeks
Chloroform	280	0.2	12.5
	140	0.1	10
	28	0.075	7.5
Control	-	0.05	5

[a] From reference Slaga and Daniel[22]

[b] Groups of 40 female SENCAR mice each were initiated with chloroform, followed by promotion with TPA (4 ug/mouse) applied once a week for 23 weeks. A chi-square test for linear trend with one degree of freedom gave a p-value of 0.25. Thus, the data does not indicate a significant increase in the proportion of mice with tumors as the result of increased exposure to chloroform.

TABLE 3. INITIATION ASSAY OF TRIHALOMETHANES[a]

GROUP	N	TREATMENT: INITIATION	TREATMENT: PART. HEP.	TREATMENT: PROMOTION[b]	GGTASE-FOCI: FOCI/CM^2
A	12	Chloroform	+	+	1.08 ± 0.28[c]
B	12	Chloroform	-	+	0.40 ± 0.21
C	12	Bromoform	+	+	0.48 ± 0.12
D	12	Bromoform	-	+	0.26 ± 0.18
E	12	Tricaprylin	+	+	1.07 ± 0.24
F	12	Tricaprylin	-	+	0.13 ± 0.07
G	12	DENA	+	+	15.8 ± 1.9

[a] From reference Pereira et al.[24]

[b] Promotion was accomplished with 500 ppm sodium phenobarbital in drinking water for 47 days

[c] Results are means ± standard errors

TABLE 4. PROMOTION ASSAY OF CHLOROFORM[a]

TREATMENT				BODY	LIVER		GGTase-FOCI
GROUP	N	INITIATION	PROMOTION	GM	GM	ORGAN/BODY WEIGHT X 100	FOCI / CM^2
A	15	WATER	TRICAPRYLIN	498 ± 12[a,c]	15.1 ± 0.5	3.0 ± 0.06	0.45 ± 0.14
B	16	DENA	TRICARPRYLIN	498 ± 10	15.4 ± 0.5	3.0 ± 0.13	0.90 ± 0.31
C	15	WATER	CHLOROFORM	466 ± 8.6	15.0 ± 0.5	3.2 ± 0.05	1.42 ± 0.44
D	16	DENA	CHLOROFORM	466 ± 9.4	14.6 ± 0.5	3.1 ± 0.08	2.22 ± 0.63[d]
E	10	DENA	BARBITAL	-	-	-	3.02 ± 1.10[d]

[a] From Pereira et al.[24]

[b] Results are means ± standard errors

[c] The initial body weights of the groups were 260-268 gm

[d] Different from group A by a non-parametric test with $p < 0.05$

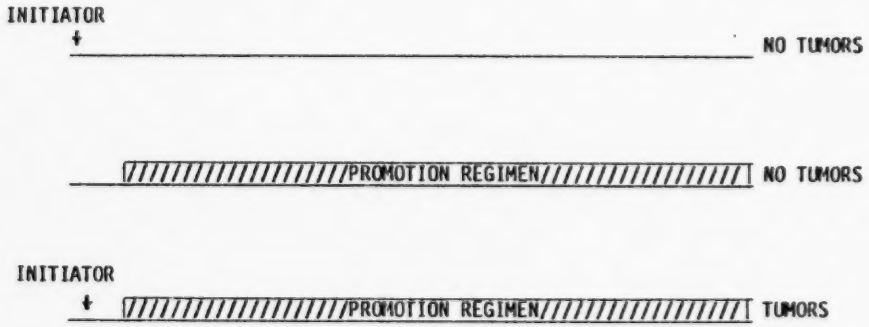
FIGURES

FIGURE 1. Schematic presentation of the protocol for initiation/promotion bioassays.

FIGURE 2. Schematic presentation of the initiation assay and promotion assay protocols of the rat liver foci bioassay.

FIGURE 3. Dose-response relationship of chloroform induction of hepatic ODC. Fisher 344 rats were injected intraperitoneally with the appropriate dose of chloroform and sacrificed 18 hours later at 9:00 a.m. Saline treated rats were used for the zero dose. All points represent the mean of a minimum of 8 animals $\pm$ SE. Results are from the four surviving rats of a group that originally consisted of eight rats. From Savage, et al.[29].

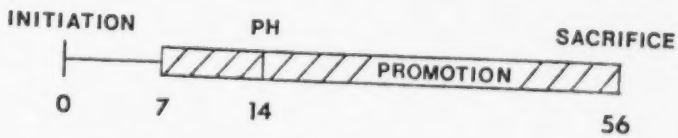
INITIATION/PROMOTION ASSAYS PROTOCOL



PROTOCOLS FOR RAT LIVER FOCI BIOASSAY

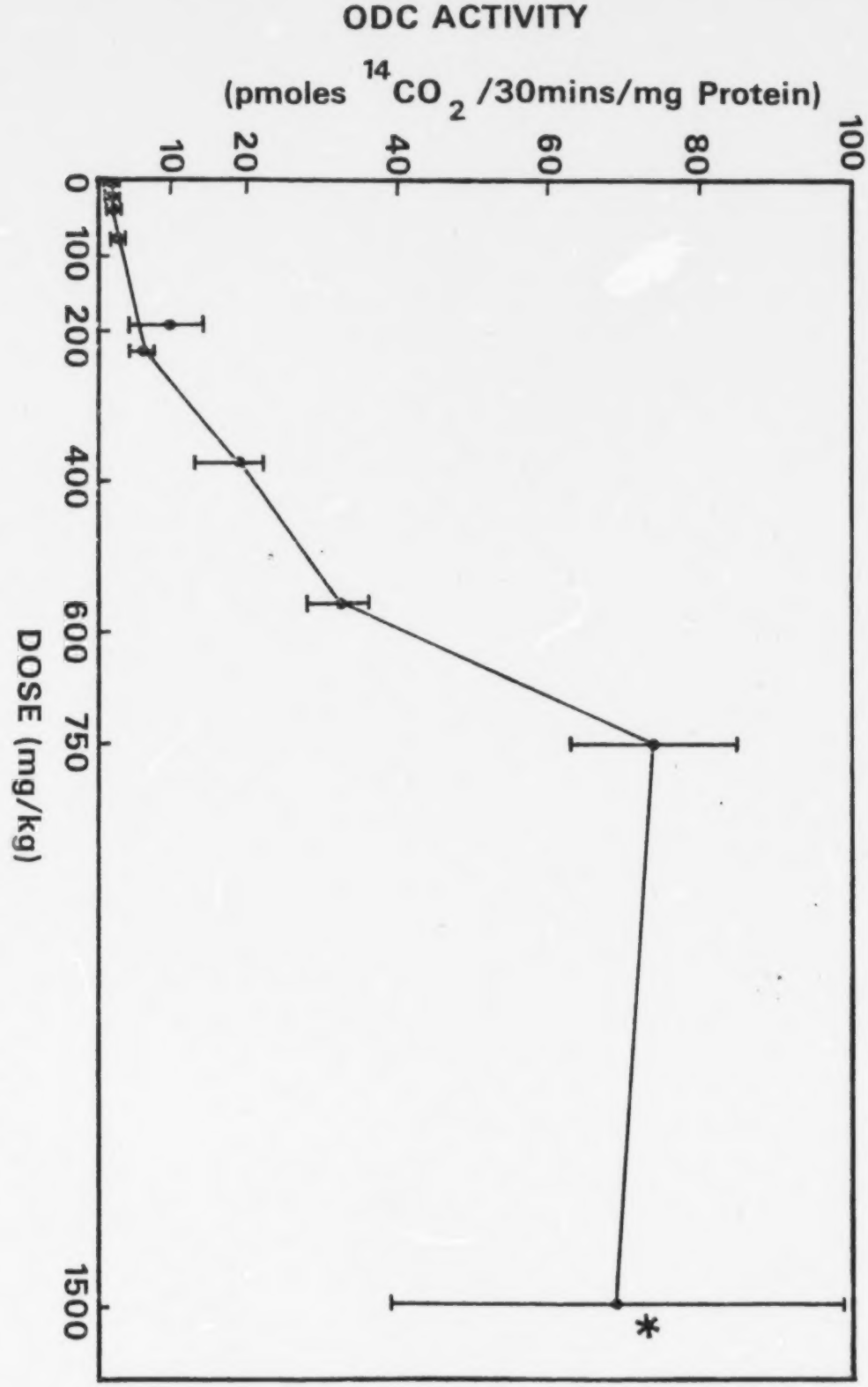
A. BROAD SPECTRUM PROTOCOL

B. 2-STAGE PROTOCOL



PH - PARTIAL HEPATECTOMY

▨ - PROMOTIOM


ODC ACTIVITY
(pmoles $^{14}CO_2$ /30mins/mg Protein)
10
20
40
60
80
100
0
100
200
400
600
750
1500
DOSE (mg/kg)
*